THE OCCURRENCE OF METHYL LÆVO-INOSITOL IN AN AUSTRALIAN POISONOUS PLANT.

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(With two Text-figures.)

(From the Physiological Laboratory of the University of Sydney.)

Heterodendron oleaefolium Desf., (Family Sapindaceæ) is a large shrub growing on the plains of the Western and Northern Interior of New South Wales, and is also found in all the other States of Australia. It has been described as a valuable forage-plant in the stock country because of its drought-resistant character.

Some time ago this plant was suspected to be the cause of certain fatalities among cattle and horses, and a sample was received by the writer for chemical investigation. It was found, when examined, to be a strongly cyanogenetic plant.

The main object of the extensive investigation carried out on this plant was, therefore, the attempt to isolate the cyanogenetic principle and to study its properties. During the course of the work there was separated a remarkable and interesting compound of lævo-inositol, and this paper will be confined to an account of the method by which it was obtained, and a general description of its characteristic features.

The material for the investigation was collected near Coonamble by Stock Inspector E. W. Procter, and forwarded to the University through the kindness of the Chief Inspector of Stock, Mr. S. T. D. Symons, M.R.C.V.S., to whom the author expresses his indebtedness and thanks.

Method of Extraction.

The plants were air-dried, and then the leaves were separated, crushed, and afterwards finely powdered. Of this air-dried leaf-powder, 2·5 kilos, corresponding to about 11·5 kilos. of fresh leaves, were extracted with 80% alcohol. The extraction was continued until almost complete; and the alcoholic fluid, measuring 31 litres, was distilled under diminished pressure, and at a temperature not exceeding 35 C. The distillation was continued till the residue was free from alcohol, and concentrated to a thick syrup. This viscous residue was treated with warm water, filtered through calico, and the residue washed until no more was dissolved. There was collected on the cloth-filter a black, sticky mass, consisting mostly of chlorophyll and resins, and weighing, when dried, 200 gms., or 8% of the air-dried leaves. The aqueous filtrate, after standing in tall cylinders for a few days, deposited a considerable quantity of brown, amorphous resin. The latter was removed and washed with cold water.

The opaque, dark brown solution was next purified by the addition (1) of an equal volume of 10% lead acetate solution, and (2) of an excess of basic lead acetate, after removing the previous bulky deposit. These very voluminous yellow precipitates were separated by spinning in the centrifuge, and were washed in the same manner with cold water, and reserved. Next, the solution was made free from lead by saturation with hydrogen sulphide, and the precipitate removed and well washed.

The aqueous solution, measuring 18 litres, was now concentrated by distillation at a low temperature, to a volume of about 2 litres. At this stage, the solution was thoroughly extracted by shaking out with ether, and in this way the free acetic acid was removed.

Crystallisation.

Two volumes of strong alcohol were then added to the solution, and, on standing to settle, a dark syrupy deposit formed, from which the solution was decanted. The latter was then concentrated by evaporation to a viscous mass, and on the addition of 95% alcohol to this residue, an insoluble viscous substance re-

mained. The alcoholic fluid was allowed to stand for some days, when there was formed a considerable quantity of clear glassy crystals. On evaporation at 35°C., and again treating with alcohol, a further separation of crystals took place. This evaporation and treatment with 95% alcohol was repeated three times, the mass of crystals was drained on a Buchner funnel, and washed with alcohol. The whole was then recrystallised from dilute alcohol, and the crystals dried. The weight of this substance, with the addition of a smaller amount obtained in the subsequent treatment of the solution, was 15 gms., equivalent to—

0·65 per cent. of the dried (at 100°) leaves.

0·60 per cent. of the air-dried material.

Purification of the crystals.—The whole of the substance was now dissolved in water, in which it was exceedingly soluble, and alcohol was carefully added to the point of incipient precipitation. On cooling the solution to 0°C., the substance slowly separated in fine transparent crystals. During this separation, the superfluid was decanted at intervals, till finally there were obtained twenty separate fractions. The first, tenth, and twentieth fractions, when dried, gave melting-points between 188° and 189·5°C. (uncorrected), thus proving the presence of a single substance only. The combined fractions were recrystallised three times, and dried in a desiccator.

Properties of the Crystals.

The following tests are described in the order in which they were performed, and show the method by which the constitution of the compound was gradually elucidated.

Preliminary tests.—(1) On fermentation with a very active preparation of emulsin, no hydrocyanic acid was evolved. The compound is, therefore, not the active principle of the plant.

(2) When heated, the substance melted, charred, and burned entirely away without residue.

It consisted of the elements carbon, hydrogen, and oxygen only. The crystals possessed a very sweet taste, and were excessively soluble in water; from which facts it may be inferred that the compound contains a number of hydroxyl groups. When

examined by the microscope, the crystal form was similar to that of cane sugar.

Molisch's reaction with sulphuric acid and α-naphthol or thymol gave no colour, and Fehling's solution was not reduced. The compound is, therefore, not a sugar.

(3) The melting-point, taste, and crystalline form are identical with those of the hexahydric alcohol, dulcitol; the latter, however, yields mucic acid when oxidised with nitric acid, whereas no mucic acid could be obtained from this substance.

(4) By treatment of the solution with phenylhydrazin acetate, no hydrazone or osazone could be obtained.

Silver nitrate in ammoniacal solution gave no precipitate in the cold; but, on warming, the solution slowly darkened with precipitation of the silver.

On boiling with dilute acids and alkalies, and subsequently recovering the compound, no apparent change in its properties was noted.

(5) *Quantitative determinations.*—The crystals, which had been formed in dilute alcoholic solution, and dried in a desiccator at the ordinary temperature, were heated at 110°C. for two hours, but showed no decrease in weight, and then at 150°C. for 30 minutes, with a similar result. The substance, therefore, contains no water of crystallisation.

The solubility showed that 1 gm. required 1·9 c.c. of water at 21°C., or 53%.

(6) The melting-point, as carefully determined on a standard Anschütz thermometer wholly immersed, was 190°C.

(7) Ultimate analysis of the substance yielded the following results:—

0·1262 gm. gave... 0·083 H_2O and 0·1983 CO_2.
Equivalent to ... 7·3% H and 42·9% C.
$C_7H_{14}O_6$ requires 7·2% H and 43·3% C.

This formula, which conforms most closely to the figures obtained for the substance, is possessed by the methyl-hexoses, simple glucosides, and certain derivatives of benzene.

(8) A determination was made of the number of methoxy

groups present in the molecule, by Perkin's modification of the method of Zeisel. By heating in pure hydriodic acid, methyl iodide distilled over into silver nitrate solution.

0·2 gm. gave ... 0·2315 gm. silver iodide.
Representing ... 0·0306 gm. CH_3O.
$C_7H_{14}O_6$ requires 0·0310 gm. for one CH_3O group.

The substance can, therefore, be represented by the formula $CH_3 \cdot C_6H_{11}O_6$, and the residue remaining in the Zeisel apparatus should possess the formula $C_6H_{12}O_6$. In order to recover this substance for examination, the solution, from which the methyl iodide had been driven off, was heated on the water-bath to remove hydriodic acid, and then evaporated to dryness. By extracting this residue with alcohol and cooling to 0°C., white crystals were recovered.

Crystals dried in desiccator and weighed... 0·160 gm.
Crystals dried at 110°C. and weighed ... 0·160 gm.
$C_7H_{14}O_6$—0·2 gm. requires ... ... 0·186 gm.

The crystals are, therefore, without water of crystallisation. The low yield may be accounted for by partial decomposition during the boiling with hydriodic acid, as the odour of benzene and phenol was distinctly detected.

(9) *Properties of the demethylated substance.*—After three crystallisations, the substance gave a melting-point of 238°C., and charred at 239°C., carefully determined on an Anschütz standard thermometer with the column submerged.

This substance also gave a negative result with Molisch's test, proving the absence of all open-chain carbohydrates; and since benzene and phenol were identified as decomposition-products of the ester, the possible cyclic compounds may next be considered.

The formula $C_6H_{12}O_6$ is contained in the inositol ring, for the identification of which the following reactions are specific:—

(*a*) *Scherer's test* gave positive reactions with this substance, and likewise with the original methyl derivative. When a little of the solution is evaporated with nitric acid, neutralised with ammonia, and calcium or barium chloride added, a brilliant rose-red colour appears. (Liebig's Annalen der Chemie u. Pharm. 81, 1852, 375).

(*b*) *Gallois' test* gave positive results with both substances. When the crystals are treated with mercuric nitrate, a yellow precipitate first forms, which, on evaporating to dryness, gives a deep red colour; on cooling, this colour slowly disappears, to return on being reheated. (Fres'. Zeitschrift für anal. Chemie, iv., 1865, 264).

These two characteristic reactions are due to the oxidation of inositol to a quinonoid substance known as rhodizonic acid, whose salts with calcium, barium, and mercury, possess the bright colours described.

The reduced substance is thus proved to be one of the inositols, and the original compound isolated from the plant-extract is its methyl ester.

(10) *Optical properties.*—A polarimetric determination of the two substances was made with a Schmidt and Haensch polarimeter reading to one-hundredth of a degree.

Methyl inositol 0·5 gm. was dissolved in 10 c.c. of distilled water at 16°C., and a lævo rotation was recorded of $-4{\cdot}01^{\circ}$ in a 1 dcm. tube.

The specific rotatory power $[\alpha]_D^{16} = -80{\cdot}2$; $[M]_D^{16} = -155{\cdot}6$.

The solution was boiled for two minutes, and after cooling to 16°C., was again read in the polarimeter. No change was observed, such as is due to mutarotation among the hexoses.

Inositol, the de-methylated compound, 0·0741 gm. was dissolved in 10 c.c. of water at 16°C., and showed a lævo rotation of $-0{\cdot}48^{\circ}$ in a 1 dcm. tube.

Specific rotatory power $[\alpha]_D^{16} = -64{\cdot}8$; $[M]_D^{16} = -116{\cdot}7$.

(11) *Hydration.*—The lævo-inositol was obtained by crystallisation from cold aqueous alcohol, and contained no water of crystallisation. When crystallised from water, it was also obtained in anhydrous crystals.

Now Maquenne and Tanret have described some important differences with regard to the water of crystallisation in the isomeric inositols.* They found that—

* Recherches sur l'inosite, Maquenne—Annales de chemie et de physique, xii., 1887, 94; Comptes rendus, cx., 1890, 87.

Inactive-inositol	
from dil. alcohol, or water under 50°C., gave ...	crysts. with $2H_2O$.
from dil. alcohol, or water above 50°C., gave ...	anhydrous crysts.
Dextro-inositol	
from dil. alcohol, or cold water, gave	anhydrous crysts.
from cold water seeded with hydrate crysts., gave	crysts. with $2H_2O$.
from hot water, gave	crysts. with $2H_2O$.
Lævo-inositol	
from cold water, gave	crysts. with $2H_2O$.
from hot water, gave	crysts. with $2H_2O$.
from dil. alcohol, gave	anhydrous crysts.
Dl-inositol	
from cold water, gave	anhydrous crysts.

It is to be pointed out that the inactive and dextro forms give reverse results with the same treatment. Maquenne, in comparing the dextro- and lævo-isomers, could always obtain the former from cold water in anhydrous crystals, but was quite unable to obtain the same with the lævo form.

The following results were obtained with the lævo-inositol from *Heterodendron*, and are of interest when compared with the figures in the previous table.

	gm.
1. Heated at 110°C. for 1 hour	0·5505
2. Dissolved in cold water, dried in desicc. at 25°C. for 2 days ..	0·5522
,, ,, ,, 25°C. for 1 day ...	0·5511
,, ,, ,, 25°C. for 1 day ...	0·5508
3. Dissolved in water at 70°C., crystd. at 70°C., dried in desicc.	
at 25°C. for 2 days	0·5678
at 25°C. for 1 day	0·5650
at 15°C. for 3 days	0·5530
at 15°C. for 3 days	0·5512
4. Heated at 100°C. for 3 hours	0·5508
5. Diss. in water, dried in the open at 15°C.	0·5508
Inositol $2H_2O$ requires	0·5660

The crystallisation from cold water, therefore, left anhydrous inositol when kept over sulphuric acid, or dried in the open.

When crystallised at 70°C., and subsequently kept over sulphuric acid for two days, the crystals contained an equivalent of two molecules of water; but since this water was gradually lost at the ordinary temperature standing over sulphuric acid, or

in the open, apparently it was not combined as hydrate in the crystals.

The hydrates obtained by Maquenne and others were only decomposed at 100°C. If the hydrate exists in the above case, it is decomposed by drying at the ordinary temperature.

The lævo-inositol of this research, therefore, was obtained in anhydrous crystals only. The hydrates prepared by the French chemists could not be obtained.

The substance isolated from *Heterodendron oleæfolium* is thus proved to be the methyl ester of *lævo-rotatory* inositol.

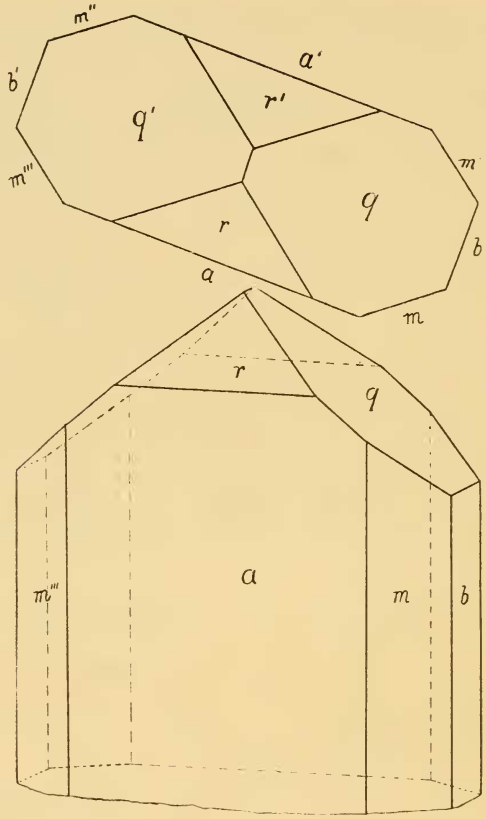
(12) *Crystal Form.*—The outward structure and measurements of the crystal forms are intimately related to the internal structure of the isomeric molecules, and, therefore, form an essential part in the elucidation of the individual members of a group. The methyl inositols apparently have never been examined by crystallographers, and indeed, as far as the author can ascertain, only inactive inositol crystals have been examined by the goniometer.

The goniometric determinations of the crystal forms of l.-methyl inositol were kindly made by Dr. C. Anderson, Mineralogist to the Australian Museum, and are here included.

Crystal Measurements of Methyl lævo-inositol.

By Charles Anderson, M.A., D.Sc.

The crystals are small, the largest being about 2 mm. in length. They belong to the orthorhombic system and are very uniform in development and habit; of the five crystals measured, four show the forms $a(100)$, $b(010)$, $m(110)$, $q(011)$, while one has, in addition, one face of the form $r(101)$, and they are all tabular on a. The faces are by no means perfect, being interrupted and wavy, the signals are only fair, and, consequently, the measurements are not in close agreement. The crystals were measured on a two-circle goniometer, the reducing lens being used.



Text-fig. 1.

The axial ratios were calculated from the following angles.

Form.	ϕ		ρ		No. of obs.
	Mean.	Limits.	Mean.	Limits.	
m110	52°44′	51°30′—53°27′	—	—	17
q011	—	—	39°26′	39°0′—39°49′	8

The elements deduced from these angles are

$$a:b:c = 0{\cdot}7609:1:0{\cdot}8224.$$

Forms and angles.

Form.	Symbol.	Measured.		Calculated.	
		ϕ	ρ	ϕ	ρ
a	100	89°54′	90°4′	90°0′	90°0′
b	010	0°5′	90°22′	0°0′	90°0′
m	110	52°44′	90°2′	—	90°0′
q	011	0°0′	39°26′	0°0′	—
r	101	88°33′	47°43′	90°0′	47°13′

The Inositols.

Position of the group.—The relative position of the group, and the mode of occurrence of its members in nature, are of considerable interest to the biochemist, especially since the discovery of "phytin" in plants by Paladin, in 1895.

The basis of inositol is the hexamethylene ring $(CH_2)_6$. Hexamethylene, C_6H_{12} (Text-fig. 1), occurs only in the hydrocarbons of the petroleum of Russia, Galicia, Baku, East Indies, and California, in the fraction boiling about 80°C. It has not been detected in plants or animals.

By the substitution of hydroxyl groups (OH) in the hexamethylene ring, the following series of compounds is obtained:—

(OH), $(OH)_2$, $(OH)_3$—synthetic compounds only.

$(OH)_4$—betite, isolated from beet sugar residues.

$(OH)_5$—quercite, in oak and other plants.

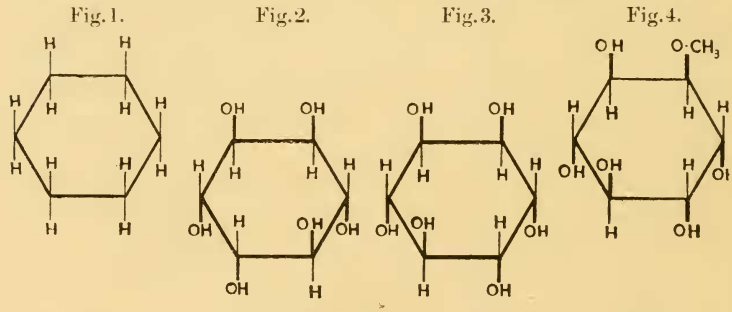
$(OH)_6$—inosite, in many animals and plants.

Inosite, or inositol, has, therefore, the constitution of a hexahydroxy hexamethylene $C_6H_6 \cdot (OH)_6$, and although its formula may be written $C_6H_{12}O_6$, it is nevertheless, in its relationships, far removed from the carbohydrates.

The constitution of Inositol.—The configuration of the inositol molecule, or, in other words, the arrangement of its atoms in space, admits of eight different geometrical groupings resulting in eight possible isomeric forms. When these and their mirror-images are built up in models, it is found that seven of the forms may be superimposed on, and, therefore, coincide with, their

mirror-images. This condition, arising from a certain degree of molecular symmetry, is accompanied by inactivity towards polarised light. These seven forms having their asymmetric carbon atoms internally compensated, are, therefore, optically inactive in the polarimeter. One form alone is found to possess an entirely asymmetric molecule, and, in consequence, this arrangement can exist as active dextro and lævo compounds, and, in addition, their *dl*- or racemic inactive combination may also exist.

Constitution of the methyl esters.—As has just been stated, the active forms of inositol are the result of one particular arrangement of the hydroxyl groups. This arrangement, which may be readily discovered in the models, is that where the six hydroxyl groups occupy the positions 1, 2, 4, on each side of the ring. This form (Fig.2) and its mirror-image (Fig.3) constitute the dextro- and lævo-inositols.



Text-fig.2.

The methyl ester, $C_6H_6(OH)_5(OCH_3)$ is obtained by substitution of a methyl group in one of the hydroxyl groups, and again from the models it can be proved that substitution in the hydroxyl 1, 2, or 4 results in three possible and different compounds being obtained. The first of these is represented by Fig.4. The corresponding three positions in the mirror-image (Fig.3)—which are identical with the alternate three positions below in the other form (Fig.2)—produce their optical anti-

podes. The possible existence of three dextro- and three lævo-methyl esters is thus determined. It now remains to ascertain (*a*) whether the few inositol esters which have been isolated up to the present time, represent the same stereo-isomer, (*b*) whether the compound obtained from *Heterodendron* is identical with any of the others, or represents the second or third isomer.

Occurrence in nature.—It has been previously stated that theoretically there can exist ten stereo-isomeric forms of inositol as a maximum possible number:—

7 inactive by internal compensation (meso),
2 active, dextro and lævo,
1 inactive, racemic or *dl*,

10 isomeric forms.

Only the inactive inositols have yet been found existing in the free state in nature, but esters of both active and inactive inositols are found.

(*a*) *Inactive inositol.*—This form, widely distributed in animal tissues, and already well known to physiologists, possesses the formula of one or other of the seven internally compensated molecules, and it is worthy of note that this is the only form found in the animal kingdom. It has always been referred to in physiological chemistry as one substance, with definite and constant general properties. But no one so far has troubled to examine minutely, material from widely different sources or organs, as to the particular properties which would differentiate these inactive isomers, such as crystallographic measurements, or optical properties.

It was discovered in animals, in 1850, by Scherer, in extracts of flesh,* and in plants, six years later, by Vohl.† This author was examining the sap of unripe pods of *Phaseolus vulgaris*, and after completely fermenting the sugars, and distilling off the alcohol, he found that the solution still possessed a very sweet taste. He then separated a manna-like substance, which he called

* Liebig's Annalen der Chemie und Pharm., lxxiii., 1850, 322.
† *Ibid.*, xcix., 1856, 125.

phasco-mannite. In the following year, the same chemist proved the identity of his mannite with Scherer's inosite from animals.

The occurrence has been recorded of three other substances, which are believed to be isomeric with inactive inositol, since they, although differing widely in crystalline form, melting-point, and solubility, possess the same general characters. These are the scyllite of Stædeler, from certain elasmobranch fishes, the quercinite of Delachanel, from the oak, and the cocositol of Mueller, from the cocoanut.

The compounds of inactive inositol which have been found in nature are:—

Bornesite—the methyl ester, obtained from caoutchouc.

Dambonite—the dimethyl ester, obtained from caoutchouc.

Phytin—the phosphate ester, an essential constituent of all plants and animals.

(*b*) *Dextro-inositol* occurs only as the methyl ester, pinite. It was discovered by Berthelot,* in 1856, in the resins from Oregon pine, and has since been found in senna leaves, and caoutchouc.

(*c*) *Lævo-inositol* is likewise found only as the methyl ester, and the following is a complete record of its occurrence:—

1. In quebracho bark, *Aspidosperma quebracho* (Apocynaceæ), discovered by Tanret, of Paris, in 1889,† and named by him quebrachite.

2. In *Hevea brasiliensis* (Euphorbiaceæ), in the aqueous solutions of the latex after coagulation of the rubber,‡ and in Para rubber.§

3. In *Grevillea robusta* (Protcaceæ).‖ It is associated in the leaves with the glucoside arbutin.

4. In *Heterodendron oleæfolium* (Sapindaceæ), this paper.

(*d*) *Racemic inositol* was discovered in mistletoe by Tanret in

* Annales de chimie et de physique, xlvi., 1856, 66.

† Comptes rendus de l'Acad. des Sciences, cix., 1889, 908.

‡ de Jong, 1906, thro. Wehmer's "Die Pflanzenstoffe."

§ Pickles and Whitfield, Proc. Chem. Soc. Lond., 1911, 54.

‖ Bourquelot et Fichtenholz, Journ. pharm. et de chimie, Paris, vi., 1912, 346.

1907.* It was found to exist free in the berries and leaves, and associated with much (meso) inactive inositol, but no active isomers were present.

The Active Forms of Inositol.

The following Table shows the physical constants by which the active forms and their compounds are identified.

	melting-point.	spec. rot. power.
(1) *Dextro-methyl ester.*		
From Pinus (Maquenne)	186°C.	+65·5
Pinus (Combes)	186·5	65·7
caoutchouc (Combes)	187	66
caoutchouc (Girard	181	64·7
(2) *Lævo-methyl ester.*		
From Quebracho (Tanret)	190°C.	−80
Rubber (Pickles and Whitfield)	191-2	80
Grevillea (Bourquelot)	190	80·3
Heterodendron (this paper) ...	190	80·2
(3) *Dextro-inositol.*		
From pinite (Maquenne)	247°C.	+65
pinite (Berthelot)	245	—
caoutchouc (Combes)	246	67·6
caoutchouc (Girard)	235	64·7
(4) *Lævo-inositol.*		
From Quebracho (Tanret)	238°C.	−65
Quebracho (Maquenne) ...	247	65
Rubber (Pickles and Whitfield)	237	—
Grevillea (Bourquelot)	247	65
Heterodendron (this paper) ...	238	64·8

Table showing the amount of methyl lævo-inositol obtained from the different sources:—

Aspidosperma quebracho ...	0·1% of dried leaves.
Hevea brasiliensis rubber ...	(2·5% of the rubber).
Grevillea robusta	0·4% of dried leaves.
Heterodendron oleæfolium ...	0·65% of dried leaves.

From the first Table, it is apparent that the lævo-methyl inositols (2) from the four different sources, have identical melting-points and specific rotatory powers, and therefore, in all probability, represent one only of the three possible stereo-isomers previously mentioned.

* Comptes rendus de l'Acad. des Sciences, cxlv., 1907, 1196.

When converted to lævo-inositol (4), however, there would appear to be two groups of melting-point figures, one 10° higher than the other, but since there can be only one possible *l*-inositol, this difference must be otherwise explained.

The Table also shows that while the dextro- and lævo-inositols (3 and 4) are optical antipodes of one another, their esters (1 and 2) are not. The optical properties especially are so very divergent that, in all probability, the methyl group occupies a different position in the two compounds. The compound isolated from Heterodendron is, therefore, shown not to be an optical isomer of Maquenne's pinite.

Biochemical Relationships and Significance.

(*a*) *The chemical aspect.*—Since the researches of Maquenne, cited in the previous paragraphs, no subsequent work has shown any relationship between the inositols and the carbohydrates, other than the sweet taste and the molecular formula common to both. Perhaps one exception to this is found in Neuberg's identification of furfural among the products of decomposition, when inositol is boiled with acids.* Although furfural is also obtained from the hexoses and heptoses in small amounts (about 0·2%), it is characteristic of the pentose sugars. It must be also remembered that the production of furfural is the basis of Molisch's group-test for all carbohydrates, and with this reagent the inositols gave negative results.

However, it seems probable from the results of many workers that the hexamethylenes form a kind of stepping-stone between the open chain compounds and the true benzene ring derivatives.

Open chain comps.	*Closed ring comps.*	
hexose sugars	*hexamethylene derivs.*	*Benzene derivs.*
dulcitol	quercitols	phenols
mannitol	inositols	
sorbitol		

The hexamethylene derivatives are much more easily decomposed than the simple benzene compounds. In fact, it has been

* Biochem. Zeitschrift, ix., 1908, 551.

found by Drechsel that fungi and bacteria may be grown in solutions of the former, especially the oxidised forms quercitol and quinic acid. It has also been proved that when phenol is exposed for some time to the alternating electric current, it is first converted to hexamethylene derivatives, before being finally oxidised and split up into various fatty acids.*

Though there are many instances of the closing up of open chain carbon compounds into ring compounds, such as the conversion of citral into cymol, the cyclo-citrals, and terpenes; geraniol into dipentene, etc.; yet no such conversions have been accomplished between the carbohydrates and the inositols Griffin and Nelson, in their researches on inositol and pinite, tried by various methods to close up the hexose chain, and to open out the inositol ring, but were entirely unsuccessful.†

(*b*) *The biochemical aspect.*—On the other hand, in favour of the biochemical possibility, there exist the important observations of Neuberg: that quercitol and inositol may be reduced in a few minutes to open chain carbohydrates (reducing Fehling's solution, etc.) by the action of sunlight and a catalyser such as uranium salt, also by the action of the alternating current.‡

These processes, however, are all reverse reactions, resulting in cleavage of the hexamethylene ring. Concerning the direct synthesis—carbohydrate to inositol, we have no evidence at all, and Maquenne had no experimental basis for his belief that the alcohol mannitol was the source of inositol.

Rosenberger observed the appearance of inositol in post mortem tissues, where previously no inositol existed; and he assumed the pre-existence of an "inositogen" from which, by enzyme-action, the inositol was formed.§

The inactive inositol combines with inorganic phosphates, and in this form exists as "phytin" in nearly all living organisms. This substance is always accompanied by the enzyme phytase,

* Journ. für prakt. Chemie, xxxviii., 1888, 65.

† Journ. Amer. Chem. Soc., xxxvii., 1915, 1552.

‡ Biochem. Zeitschrift, xiii., 1908, 308.

§ Zeitschrift für physiol. Chemie, lvi., 1908, 373.

which effects its cleavage and yields inositol again, in the free state. Starkenstein's investigations show that the source of free inositol in tissues is the phytin, and that inositol is a decomposition-product of the phosphoric acid metabolism in both plants and animals.* Indeed, much work has been done in elucidating the conditions of this transformation on the side of the phosphoric acid, but again, as to the inositol side, nothing is known.

The few definite observations concerning the part played in metabolism, by inositol, are here summarised:—

1. In *unripe seeds*, inositol and quercitol accumulate just at the time when the transport of carbohydrates to the fruit begins.

2. As the *fruit ripens*, Vohl observed that inositol and quercitol disappear, and are changed into "phytin" (not carbohydrate).

3. On the *germination of the seeds*, inositol again makes its appearance, both when grown in the dark and in the light.

4. During the metabolism of the *growing plant*, inositol disappears gradually with the rest of the reserve-substances.

Thus it comes in at the beginning and later passes out again, without a clue to its precursors or katabolites.

When fed to animals, or injected into the blood-stream, inositol is about three-fourths decomposed, and the remainder may be recovered from the urine unchanged. Mayer injected large doses into rabbits, and obtained, from the urine, racemic lactic acid. It is likewise decomposed by fungi into butyric and lactic acids.

This inactive inositol, which occurs so widely in fresh green plants, has been shown by many workers to be present in much larger quantities in young growing plants (and animals) than in the adult forms. It almost entirely disappears from plants when they are slowly dried.

The esters of active inositol, on the other hand, do not vanish on drying the plants. When we consider the great rarity of their occurrence, and the fact that the active forms have never been identified in nature as free inositol, it almost leads one to assume for them a different origin. Such an origin would be more in common with that of certain well known plant-con-

* Biochem. Zeitschrift, xxx., 1911, 98.

stituents, which also possess side-chains in the 1. 2. 4. positions on the benzene ring, corresponding to the positions of the hydroxyl groups of the active inositol esters: a few of these may be mentioned, such as vanillin, eugenol, safrol, coniferyl alcohol, protocatechuic and caffeic acids.

In conclusion, the author desires to express his indebtedness to Professor Sir Thomas Anderson Stuart, in whose laboratory this work has been done.

Summary.

The endemic Australian plant, *Heterodendron oleæfolium* Desf., Family Sapindaceæ, contains the methyl ester of lævo-rotatory inositol.

The amount isolated was equivalent to 0·65% of the dried (at 100°C.) leaves.

This substance is not optically isomeric with the pinite of Maquenne, which is the methyl dextro-inositol, possessing a different melting-point and optical rotation.

It is apparently identical with Tanret's quebrachite, and has been previously recorded from three plants only—*Aspidosperma quebracho* (Apocynaceæ), *Hevea brasiliensis* (Euphorbiaceæ), and *Grevillea robusta* (Proteaceæ).

The occurrence of this compound is, therefore, exceedingly rare, and is in great contrast to the occurrence of *inactive* inositol, which exists as a plastic substance in most plants.

Heterodendron also contains a cyanogenetic glucoside.